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## RESEARCH ARTICLE

## Salt preferences of honey bee water foragers

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## ABSTRACT

The importance of dietary salt may explain why bees are often observed collecting brackish water, a habit that may expose them to harmful xenobiotics. However, the individual salt preferences of water-collecting bees were not known. We measured the proboscis extension reflex (PER) response of *Apis mellifera* water foragers to 0–10% w/w solutions of Na, Mg and K, ions that provide essential nutrients. We also tested phosphate, which can deter foraging. Bees exhibited significant preferences, with the most PER responses for 1.5–3% Na and 1.5% Mg. However, K and phosphate were largely aversive and elicited PER responses only for the lowest concentrations, suggesting a way to deter bees from visiting contaminated water. We then analyzed the salt content of water sources that bees collected in urban and semi-urban environments. Bees collected water with a wide range of salt concentrations, but most collected water sources had relatively low salt concentrations, with the exception of seawater and swimming pools, which had >0.6% Na. The high levels of PER responsiveness elicited by 1.5–3% Na may explain why bees are willing to collect such salty water. Interestingly, bees exhibited high individual variation in salt preferences: individual identity accounted for 32% of variation in PER responses. Salt specialization may therefore occur in water foragers.

**KEY WORDS:** *Apis mellifera*, Water foraging, PER, Sodium preference, Salt concentration

## INTRODUCTION

Honey bees collect water for multiple reasons: colony temperature regulation (Kühnholz and Seeley, 1997), metabolic needs (Louv and Hadley, 1985) and larval food (Nicolson, 2009). In honey bees, sodium, magnesium and potassium are essential for developing larvae (Herbert et al., 1978), and salts obtained from water may therefore be an essential part of the brood food provided by nurse bees (Brodschneider and Crailsheim, 2010). In general, insects such as bees can obtain salt from multiple sources: human tears, carrion, feces or brackish water (Abrol et al., 2012; Baumgartner and Roubik, 1989; Bänziger et al., 2009; Ferry and Corbet, 1996). Honey bees seem to prefer agricultural or urban water runoff, perhaps because it is common and contains salts (Butler, 1940; Hooper, 1932). However, these preferences are poorly understood.

Butler (1940) showed that honey bees have strong group-foraging preferences for water with specific salt concentrations. Because foraging bees exhibit social facilitation, they are attracted to the presence of other bees (Avarguès-Weber et al., 2015), and thus it is

difficult to disentangle group foraging preferences from individual preferences. Since Butler (1940), there has been little progress in understanding the salt preferences of water foragers. Research with nectar foragers has shown that a sufficiently high salt concentration is a punishing stimulus (Abramson, 1986; Bhagavan and Smith, 1997; Letzkus et al., 2006), and that nectar foragers can be repelled by higher concentrations of potassium and phosphate in nectar (Afik et al., 2006; Hagler, 1990; Waller et al., 1972). However, the salt preferences of individual water foragers are not known.

Understanding these salt preferences is important for understanding honey bee biology and, potentially, for developing salt additives to deter bees from collecting agricultural water with harmful xenobiotics. Honey bees are essential for the pollination of many crops (Klein et al., 2007), but the widespread use of agricultural toxins and pesticides can decrease honey bee health (Blacqui re et al., 2012; Desneux et al., 2007; Goulson et al., 2015). Honey bee pesticide exposure is typically thought to occur through nectar and pollen consumption, but pesticides also occur in agricultural runoff (Goulson, 2013; Sanchez-Bayo and Goka, 2014), and the application of systemic pesticides by watering plant roots can result in high water-borne pesticide concentrations (Johnson and Pettis, 2014; Phillips and Bode, 2004; Samson-Robert et al., 2014). Bees collecting such runoff water place themselves and their colonies at risk.

Our goal was therefore to study individual salt preferences in honey bee water foragers and determine which concentrations are attractive and aversive. We used the proboscis extension reflex (PER) assay, which is widely used to study bee sucrose response thresholds and has successfully elucidated multiple aspects of individual and colony behavior (Page, 2013). In this assay, the investigator taps the bee's antennae, which are rich in salt and sugar receptors (de Brito Sanchez et al., 2014). The bee reflexively extends its proboscis to drink if the concentration is acceptable. We tested the PER response of water foragers to different concentrations of NaCl, MgCl<sub>2</sub>, KCl and Na<sub>2</sub>HPO<sub>4</sub> (phosphate) because Na, Mg and K are key bee nutrients (Herbert et al., 1978) and because phosphate can repel nectar foragers (Afik et al., 2006). In addition, we analyzed the salt concentrations of water sources that honey bees collected in urban and semi-urban settings to determine salt concentrations in bee-collected water.

## MATERIALS AND METHODS

## Study site and colonies

Between April 2012 and June 2013, we performed the PER experiment with 12 healthy colonies of *Apis mellifera ligustica* Spinola 1806 reared from packages at the UC San Diego Biological Field Station (BFS: 32°53'13"N, 117°13'48"W) in La Jolla, CA, USA. In total, we used 163 bees from 12 colonies to test the effect of salt concentration presentation order (Fig. 1) and 628 bees from 10 colonies to test bee salt preferences (Fig. 2).

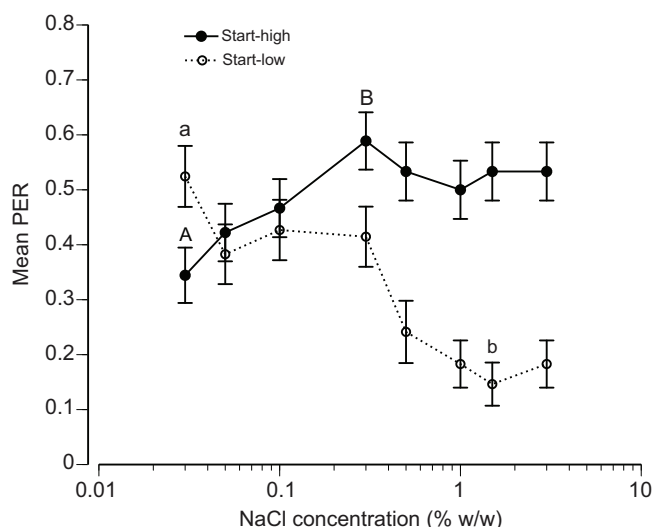
## PER measurements of honey bee salt preferences

We tested honey bee PER responsiveness to different salts. To obtain water foragers, we placed a grooved plate feeder (design of

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**Fig. 1. Effect of the order of NaCl concentration presentation on the proboscis extension reflex (PER).** A value of 1 indicates all bees exhibited PER. Data are means  $\pm$  1 s.e.m. Different letters indicate significant differences (uppercase letters for start-high concentrations and lowercase letters for start-low concentrations, Tukey HSD tests,  $P < 0.05$ ,  $N = 163$  bees from 12 colonies).

Von Frisch, 1967) containing tapwater at the entrance of each colony. Deionized water and distilled water do not contain salts. We therefore chose tapwater as a realistic, standardized water source for bees. Chemical analysis showed that this tapwater contained the following salts: Na (0.0104%), Mg (0.0027%), K (0.0006%) and phosphate (0%). These concentrations are roughly similar to salt concentrations in freshwater sources that bees collected (see below). Guard bees did not tolerate non-nestmates foraging for water, and this allowed us to determine bee colony origin. Bees collecting water were captured in vials and brought back to the lab, where they were anesthetized at 0°C until their movements significantly decreased (1–2 min). Anesthetized bees were placed in a standard PER harness: a strip of duct tape that restrained the bee inside a 3.7 cm long  $\times$  15 mm wide stainless steel tube. This harness allowed bees to move their mouthparts and antennae (Giurfa and Sandoz, 2012). The anesthetized bees then recovered for 20 min in a 30°C incubator, followed by 10 min at room temperature (21°C).

To test the PER, we simultaneously stimulated both antennae for 3 s with a microcapillary pipette dipped into the test solution. We took care to ensure that no solution or residue built up on the antennae. We used a 2 min inter-trial interval between each test (Page et al., 1998). For NaCl, we tested the effects of concentration order (start-low versus start-high, Fig. 1). Based upon these results, we used the start-high concentration order for all subsequent tests. Like experiments that use the PER to measure bee sucrose response thresholds (Page et al., 1998), our salt reward gradient thus went from low reward to high reward. In sucrose response threshold assays, nectar foragers are presented with a low reward, water, and then with successively higher rewards, higher sucrose concentrations (Page et al., 1998). For honey bees, high salt concentrations provide a low reward because bees are attracted to low salt concentrations and are repelled by high salt concentrations (Butler, 1940).

Test solutions contained different concentrations of NaCl,  $\text{MgCl}_2$ , KCl or  $\text{Na}_2\text{HPO}_4$  (ACS reagent grade compounds,  $\geq 99.8\%$  purity, Fisher Chemical) in distilled water. We used the following concentrations of each salt: (1) 0%, 0.03%, 0.05%, 0.1%, 0.3%, 0.5%, 1%, 1.5% and 3% NaCl to test for the effect of

concentration order (Fig. 1); (2) 0%, 0.05%, 0.4%, 0.75%, 1.5%, 6% and 10% of all salts for the full-range tests (Fig. 2A); and (3) ranges individually tailored for bee PER responses to each salt concentration: NaCl (0–3%),  $\text{MgCl}_2$  (0–6%), and KCl and  $\text{Na}_2\text{HPO}_4$  (0–1.5%, Fig. 2B). These concentrations are all much lower than NaCl concentrations used as training punishment in nectar foragers: 35.7% (Abramson, 1986) and 17.53% (Bhagavan and Smith, 1997). All salt concentrations are given as w/w.

We did not provide a pure water stimulus between each salt test. Sucrose PER assays sometimes include a water stimulus between successive sucrose presentations. This water stimulus controls for increased sensitization or habituation to repeated sucrose stimulations because nectar foragers should not exhibit PER responses to a pure water stimulus (Page et al., 1998). However, in our experiment, we tested the PER response of water foragers to water with different concentrations of salts. Pure water could therefore have provided a reward, not a neutral control stimulus, particularly for salts (K and phosphate) that elicited a largely aversive response at nearly all concentrations. We consequently adopted the protocol of some PER experiments with sucrose solutions (Decourtye et al., 2004; Eiri and Nieh, 2012; Lambin et al., 2001) and did not intersperse pure water presentations between test solution presentations.

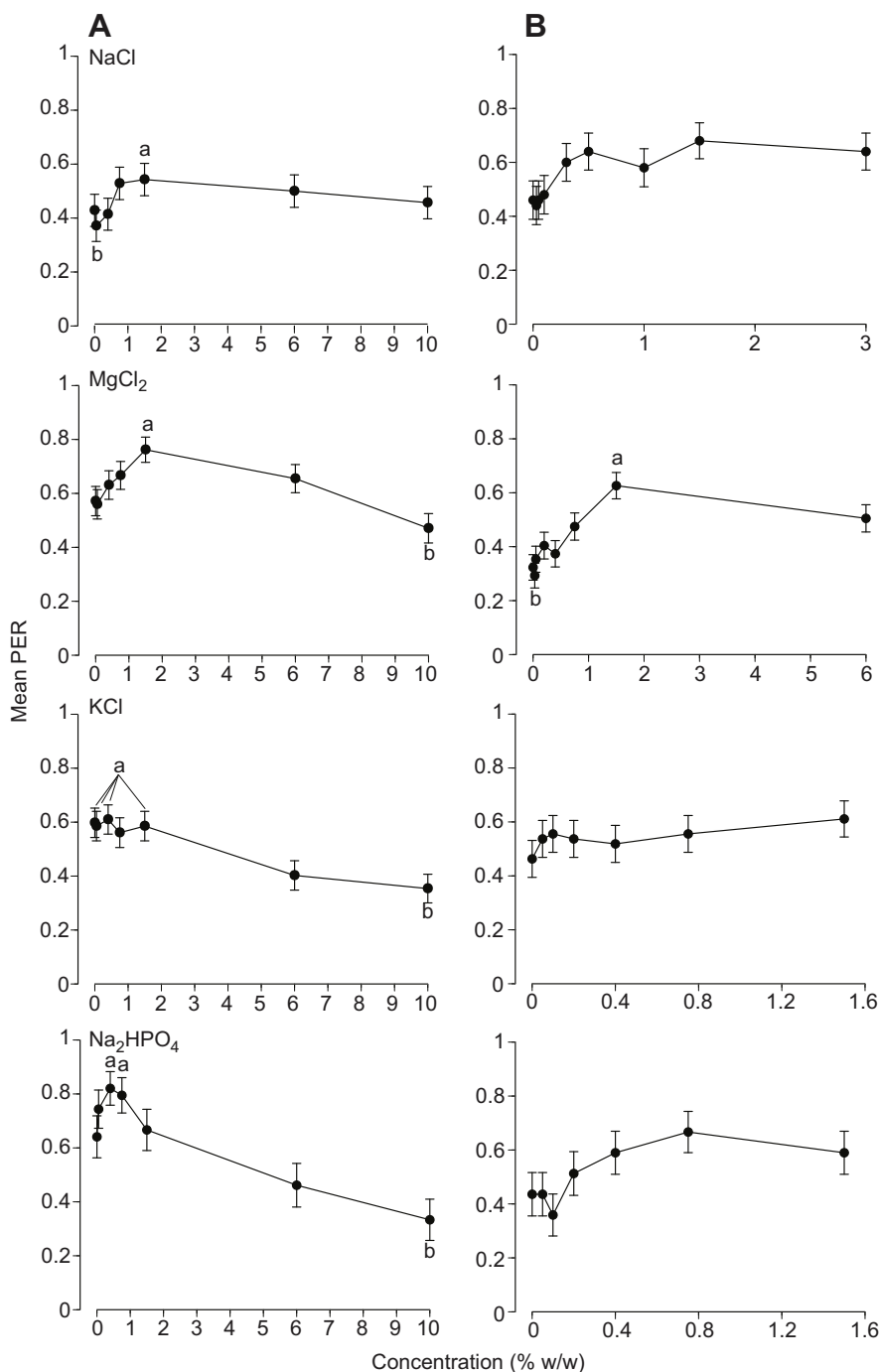
Responses were categorized as 1 (proboscis fully extended beyond the mouthparts) or 0 (no proboscis extension). Bees were kept in tubes for approximately 20–25 min during testing. After testing, bees were painted with red enamel on the thorax and released to ensure that we did not pseudoreplicate by using the same bee in subsequent trials. Each day, we ran either two or three sets of 10 bees between 09:00 h and 16:00 h.

### Water collection

Because the salinity of water sources may change over time, we only took water samples when bees were actively collecting water, defined as a bee inserting its proboscis into water for more than 10 s, often showing rhythmic abdominal contractions that characterize fluid imbibing.

To maximize water collection, we involved citizen scientists from a local beekeeping group. All water collectors were trained in how to collect samples and, with each sample, were required to submit a written behavioral description and a photo of a bee actively collecting water at the time of water collection. Samples without these written descriptions and photos were not analyzed. We collected samples in clean 50 ml conical plastic centrifuge tubes (Falcon, model no. 352070), rinsing each tube three times with the sampled water before collecting the final sample, which we froze until analysis. Collectors also recorded the type of water source and the GPS coordinates or the nearest cross-street location (Fig. 3). Samples were analyzed by the Oklahoma State University Soil, Water, and Forage Analytical Laboratory for Na, Mg, K and total phosphate with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Murray et al., 2000).

We classified water sources into seven categories. Natural sources consisted of (1) streams, (2) natural perennial ponds and (3) beach saltwater. Man-made sources consisted of (4) irrigation (leaking spigots, sprinkler heads, or temporary pools of water resulting from irrigation), (5) swimming pools, and artificial ponds that fell into two distinct size groups: (6) large artificial ponds and (7) small ‘ponds’ in vessels (bird baths, small ornamental waterfalls and fountains). Large ponds were always inset into the ground, where they received runoff water from irrigation sprinklers and rain. Small ponds were always elevated above the ground, were all  $< 1$  m in



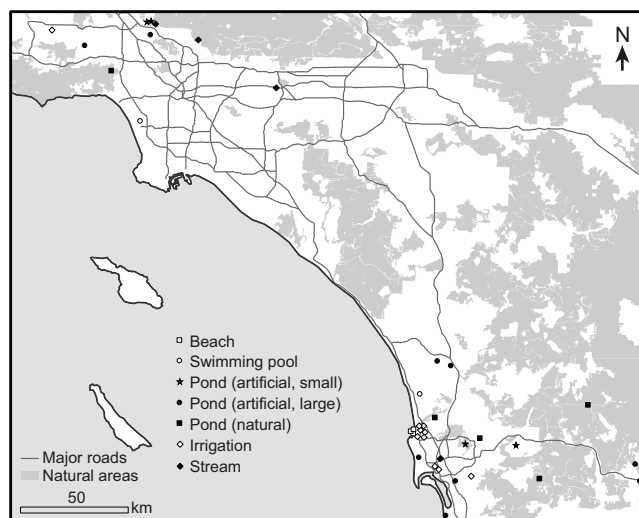
**Fig. 2. Mean PER responses to different salt solutions.** (A) In the full concentration range tests, all bees received the same concentration of each salt. (B) In the finer concentration range tests, we tested a more limited range of concentrations, tailored to each salt, to obtain more detailed information on PER responses. Data are means  $\pm$  1 s.e.m. Different letters show significant differences (Tukey HSD tests,  $P < 0.05$ ,  $N = 628$  bees from 10 colonies). All other concentrations were intermediate. If no letters are shown in a plot, there were no significant pairwise PER response differences between concentrations.

diameter, and contained water that was subject to higher rates of evaporation because it was in a shallow vessel (a bird bath) or in a small fountain that constantly recirculated water over a broad surface area. More water samples were collected from urban and semi-urban sites; however, 36% of our water samples came from natural water sources.

### Statistics

We used repeated-measures ANOVA with a REML algorithm to determine the effect of concentration order on bee PER responses with salt concentration as a continuous fixed effect and bee identity and colony as random effects. We used the same analysis method to determine the effect of salt type (fixed effect) and salt concentration

(continuous fixed effect) on bee PER responses (bee and colony identity were random effects). To determine the significance of bee identity on PER responses to the full salt concentration ranges, we also ran a model with bee identity as a fixed effect. We then tested responses to each salt separately, using a one-way repeated measures ANOVA and Tukey's honestly significant difference (HSD) tests to make pairwise comparisons (concentration coded as an ordinal fixed effect). These analyses used data gathered on bees tested with the same concentrations of each salt. Finer scale salt preferences were analyzed with one-way repeated measures ANOVA models for each salt. We did not use an overall ANOVA on these data because different concentrations were used for different salts. We used JMP v10.0 statistical software.



**Fig. 3. Sources of water collected by honey bees.** Samples were obtained from the locations shown for analysis.

## RESULTS

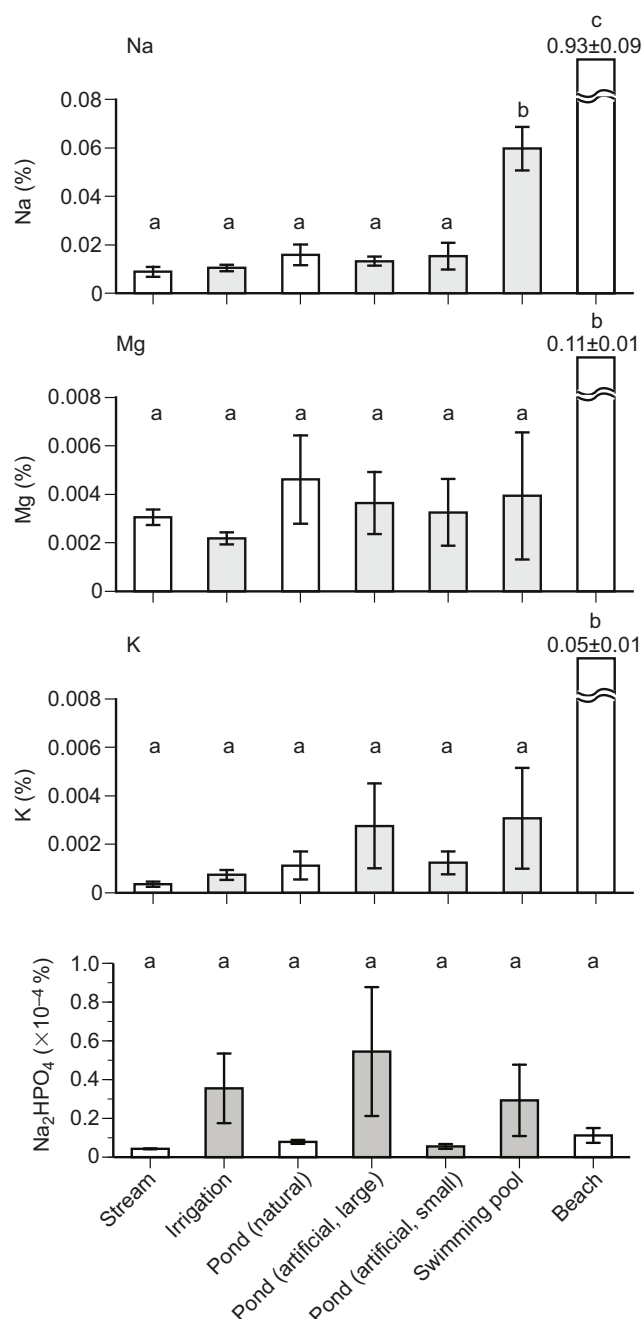
### Effect of concentration order

We presented bees with a series of Na concentrations and measured their PER responses. There was a significant effect of concentration order (start-low versus start-high) on NaCl preferences (order effect:  $F_{1,251}=26.54$ ,  $P<0.001$ ). There was also a significant effect of concentration ( $F_{8,1477}=4.19$ ,  $P<0.001$ ) and the interaction order $\times$ concentration ( $F_{8,1477}=13.24$ ,  $P<0.0001$ ), with colony accounting for 15% of model variance. The start-low bees showed a steadily declining response with increasing concentration and a slight potential peak at 0.1–0.3% NaCl, whereas the start-high bees showed a true response peak at 0.3% (Fig. 1). In terms of bee preferences, the two treatments therefore yielded roughly similar results, a peak at approximately 0.3% NaCl. However, beginning the series with a high salt concentration revealed bee preferences more clearly (Fig. 1). For start-high bees, PER responses for 0.3% NaCl were significantly higher than those for 0.03% NaCl (lowest PER response, Tukey's HSD,  $P<0.05$ ). PER responses to 0.3% NaCl were not significantly different from responses to 1.5% NaCl (Tukey HSD,  $P>0.05$ ), matching bee preferences in our subsequent tests.

### Full salt concentration range

Bees exhibited different preferences for different salts. Thus, there were significant effects of salt type ( $F_{3,228}=4.22$ ,  $P=0.006$ ), concentration ( $F_{1,1667}=51.63$ ,  $P<0.0001$ ), and the interaction salt type $\times$ concentration ( $F_{3,1667}=12.34$ ,  $P<0.0001$ ). The significant interaction is shown in the different PER response curves for each ion (Fig. 2A). There was substantial variation between bees (32.1% of model variance) but relatively little variation between colonies (0.4% of model variance). There were strong individual differences in salt preferences ( $F_{256,241}=4.02$ ,  $P<0.0001$ ).

We then examined the effect of each salt type (Fig. 2A). Mean bee PER responses were significantly higher for 1.5% NaCl, 1.5%  $\text{MgCl}_2$ , and 0.4% and 0.75%  $\text{Na}_2\text{HPO}_4$  than for a concentration that elicited the lowest PER response (concentration effects  $F_{6,498}\geq 5.17$ ,  $P<0.0001$ , Tukey's HSD tests,  $P<0.05$ ). For KCl, bee PER responses were significantly higher for concentrations of 0–1.5% (excluding 0.75%, concentration effect:  $F_{6,492}=5.26$ ,  $P<0.0001$ , Tukey's HSD test,  $P<0.05$ ).



**Fig. 4. Chemical content of water collected by honey bees.** The mean percentage ( $\pm 1$  s.e.m.) is shown. Because of the highly elevated concentrations of Na, Mg and K in seawater, we show this information above the bars for seawater. Significant differences are indicated with different letters. Gray bars indicate man-made water sources.

The 10% concentrations generally elicited the fewest PER responses: 10%  $\text{MgCl}_2$ , KCl and  $\text{Na}_2\text{HPO}_4$  resulted in significantly lower PER responses (Tukey's HSD tests,  $P<0.05$ ). Somewhat surprisingly, bees tolerated a 10% NaCl solution (Fig. 2B).

### Finer salt concentration ranges

Different salts elicited PER responses that peaked at different concentrations (Fig. 2A). We therefore tested bee responses to a more limited range of salt concentrations that were tailored to bee



PER responses (Fig. 2B). There were significant overall effects of concentration for NaCl ( $F_{8,392}=3.42$ ,  $P=0.0008$ ),  $MgCl_2$  ( $F_{7,687}=7.85$ ,  $P<0.0001$ ) and KCl ( $F_{6,228}=3.37$ ,  $P=0.0033$ ). The only significant pairwise differences (Tukey's HSD test,  $P<0.05$ ) occurred for  $MgCl_2$ , between 0.03% and 1.5% (higher PER for 1.5%, as in the full concentration range test). There was no significant effect of  $Na_2HPO_4$  ( $F_{6,324}=0.65$ ,  $P=0.69$ ) over the concentration range 0–1.5%.

### Salt concentrations in water that bees collected

We analyzed water collected by bees from 36 sites in San Diego and Los Angeles Counties (Fig. 3) from June 2012 to June 2013. In all analyses, internal control ICP-AES standards showed negligible levels of each ion (0.0 ppm). There was a significant effect of water type on the concentration of Na ( $F_{6,29}=43.0$ ,  $P<0.0001$ ), Mg ( $F_{6,29}=5.79$ ,  $P=0.0005$ ) and K ( $F_{6,29}=9.08$ ,  $P<0.0001$ ), but not on the concentration of phosphate ( $F_{6,29}=1.67$ ,  $P=0.16$ ). As expected, seawater (0.93%) and swimming pool water (0.6%) contained higher sodium concentrations than all other water sources (Tukey HSD,  $P<0.05$ ; Fig. 4). For Mg and K, only seawater had significantly elevated ion levels compared with all other water sources (Tukey HSD,  $P<0.05$ ; Fig. 4).

### DISCUSSION

Honey bees are known to collect and, in some cases, prefer 'dirty water' that contains salts (Butler, 1940). Understanding these preferences has practical importance because such water can contain harmful xenobiotics such as pesticides. Using the classic PER assay, we found differences in the responses of water foragers to the tested salts. Overall, mean PER responses to NaCl and  $MgCl_2$  were 22% higher than for  $Na_2HPO_4$  and KCl. In general, water foragers exhibited the fewest responses to 10% salt concentrations. However, water foragers demonstrated tolerance to a far wider range of Na concentrations than to other ions, perhaps explaining why honey bees can collect seawater. The concentrations that elicited the most PER responses varied somewhat depending upon the range of concentrations tested (Fig. 2). The optimal salt concentrations were 1.5% for NaCl and  $MgCl_2$ , 0–1.5% for KCl and 0.4–0.75% for  $Na_2HPO_4$  (Fig. 2A). The analysis of finer salt concentration ranges yielded similarly shaped PER curves (Fig. 2B), though fewer significant pairwise differences because we did not include the generally aversive 10% concentration.

### Responses to each salt

Bees exhibited the most PER responses for intermediate concentrations of sodium and magnesium, corresponding to the attraction found by Butler (1940). Using group-foraging experiments, Butler (1940) showed that bees had a preference for 0.15%, 0.07% and 0.29% NaCl over distilled water (maximum tested concentration of 0.58% NaCl). In our study (Table 1), bees were least responsive to 0.05% NaCl but exhibited the most PER responses to 1.5% NaCl (full-range tests; Fig. 2A). Like Butler (1940), we found responsiveness at 0.3% NaCl, but bees continued to respond at higher concentrations than he tested (Fig. 2).

Butler (1940) also tested  $MgCl_2$  concentrations up to 0.92% and found that honey bees preferred distilled water regardless of the  $MgCl_2$  concentration. In our study, there was a higher response to 1.5%  $MgCl_2$  than for distilled water.

For potassium and phosphate, water foragers exhibited the most PER responses for the lowest concentrations, corresponding to the aversion demonstrated by Butler (1940). For KCl, bees showed a diminished response above 1.5% (Fig. 2A). Similarly,

**Table 1. Review of the published concentration ranges of salt ions that attracted or repulsed bees in honey and in water and nectar visited by bees**

Salts	Concentration (% w/w)	
	Acceptance	Rejection
Na	0.0019 <sup>1,a</sup> nectar	0.0054 <sup>1,g</sup> nectar
	0.0075 <sup>2</sup> honey	0.0059 <sup>1,g</sup> honey
	0.0079 <sup>1</sup> honey	0.0130 <sup>3,b</sup> nectar
	0.0093 <sup>3,a</sup> nectar	<b>0–0.05<sup>c</sup> water</b>
	0.0226 <sup>4</sup> nectar	0.58 <sup>5,d</sup> water
	<b>0.04 water</b>	3.59 <sup>9,h</sup> water
	<b>0.05<sup>c</sup> water</b>	17.53 <sup>10,f</sup> water
	<b>0.07 swimming pool</b>	
	0.15 <sup>5,d</sup> water	
	0.39 <sup>6,d</sup> water	
	0.58 <sup>7,e</sup> water	
	<b>1.5<sup>c</sup> water</b>	
	2.34 <sup>8,f</sup> water	
	<0.0005 <sup>1,a</sup> nectar	0.0024 <sup>3,b</sup> nectar
Mg	<b>0.007 swimming pool</b>	0.0188 <sup>1,g</sup> nectar
	0.0090 <sup>2</sup> honey	0.0205 <sup>1,g</sup> honey
	<b>0.012 water</b>	0.95 <sup>5,d</sup> water
	0.0185 <sup>1</sup> honey	<b>10<sup>c</sup> water</b>
	0.06 <sup>5,d</sup> water	
	<b>1.5<sup>c</sup> water</b>	
	0.00075 <sup>9,g</sup> water	0.0659 <sup>3,a</sup> nectar
	<b>0.005 swimming pool</b>	0.15–0.75 <sup>11,i</sup> nectar
	0.0325 <sup>1,b</sup> honey	0.36–1.30 <sup>3,b</sup> nectar
	<b>0.06 water</b>	0.3768 <sup>1,g</sup> honey
K	0.1480 <sup>2</sup> honey	0.3946 <sup>1,g</sup> nectar
	0.21 <sup>5,d</sup> water	0.42–0.89 <sup>2,b</sup> nectar
	<b>0–1.5<sup>c</sup> water</b>	0.53–0.69 <sup>13,i</sup> nectar
		1.66 <sup>5,d</sup> water
		<b>10<sup>e</sup> water</b>
		0.0511 <sup>1,g</sup> nectar
		0.0652 <sup>1,g</sup> honey
Phosphate	<b>2–343 (<math>\times 10^{-6}</math>) water</b>	1.42 <sup>5,d</sup> water
	<b>11–48 (<math>\times 10^{-6}</math>) swimming pool</b>	<b>10<sup>c</sup> water</b>
	<b>0–1.5<sup>c</sup> water</b>	
	0.0019 <sup>1,a</sup> nectar	
	0.0047 <sup>1,a</sup> honey	

Concentration ranges are from minimum to maximum. Swimming pool water has higher ion concentrations and is separately denoted. Data from the present study are in bold.

<sup>1</sup>Afik et al. (2006); <sup>2</sup>McLellan (1975); <sup>3</sup>Waller et al. (1972); <sup>4</sup>Nicolson and W.-Worswick (1990); <sup>5</sup>Butler (1940); <sup>6</sup>Hooper (1932); <sup>7</sup>de Brito Sanchez et al. (2014); <sup>8</sup>de Brito Sanchez et al. (2005); <sup>9</sup>Abramson et al. (2013); <sup>10</sup>Bhagavan and Smith (1997); <sup>11</sup>Waller (1972); <sup>12</sup>Waller et al. (1976); <sup>13</sup>Hagler (1990).

<sup>a</sup>Non-avocado and non-onion nectar; <sup>b</sup>onion nectar; <sup>c</sup>PER assay (this paper); <sup>d</sup>water feeder dishes; <sup>e</sup>salt solutions applied to bee tarsomeres; <sup>f</sup>PER assay; <sup>g</sup>avocado nectar and honey; <sup>h</sup>choice between NaCl and sucrose; <sup>i</sup>sugar solution with K added.

Butler (1940) tested KI up to 1.66% and found a decreasing preference as KI concentration increased. Nectar foragers are known to avoid nectar with high levels of potassium (Afik et al., 2006; Hagler, 1990). For example, nectar foragers are reluctant to forage on onion nectar, which has up to 1.3% potassium (Table 1; see also Waller et al., 1972). Our water forager preferences (rejection >1.5% K) are similar to the preferences exhibited by nectar foragers for sugar solutions with added potassium (Table 1).

Butler (1940) reported that almost no bees visited a 1.42% solution of  $Na_2HPO_4$  (the only concentration he tested). We found that PER responses declined for concentrations above 0.75%  $Na_2HPO_4$ . Natural concentrations of phosphate are far lower (maximum of 0.05% in avocado nectar; Table 1; see also Afik et al., 2006).

### Water sources visited by bees

Surprisingly, there is little published information on the water sources collected by honey bees. Baum et al. (2011) observed honey bees collecting water from swimming pools in Tucson, AZ, USA. Beekeepers (personal communications) have witnessed bees collecting seawater, although these observations have not previously been published. In Southern California, an arid region, we found that bees visited a wide range of water sources. If we exclude swimming pools (salts deliberately added) and seawater (naturally quite salty), we found that bees collected water, on average, with 0.013% Na, 0.003% Mg, 0.001% K and 0.00003% phosphate, far lower than the values that elicit maximal attraction (Butler, 1940) or PER responses. Seawater that bees collected (0.93% Na, 0.11% Mg, 0.05% K and 0.00001% phosphate) contained more Na than group-foragers preferred (Butler, 1940), but contained salt concentrations that individually elicited high average PER responses (Fig. 2). Our results may help explain why bees collect seawater, particularly if given limited alternatives.

It is possible to categorize the water sources that bees visited in different ways, but this would not have changed our results. The only two sources that showed significant differences were seawater and swimming pool water (Fig. 4). For total phosphate, there were no significant differences, although irrigation, artificial pond and swimming pool water had the highest levels measured.

A bigger dataset on water sources visited by bees is desirable. However, we were surprised, given the large number of enthusiastic volunteers over 1 year, to have only obtained 36 samples. The relative infrequency of water foraging may play a role. In many cases, we were told of water sources that bees were observed collecting, but upon arriving, found no bees collecting water, even over several hours of observation. It would be valuable to continue collecting data on such water sources, but an experimental approach testing bee salt concentration choices is more feasible.

### Deterrence

Potassium and phosphate at higher concentrations could deter water collection by bees. Given concerns about eutrophication (Schoumans et al., 2014), adding potassium to water-delivered pesticides may be a better alternative than adding phosphate. Determining an effective concentration requires field tests, but multiple studies (Table 1) show that nectar foragers will avoid a wide range of potassium concentrations in sugar water (0.15–10%) and are reluctant to collect nectar from avocado (Afik et al., 2006) and onion blossoms (Waller et al., 1972) with high potassium content. A blend such as Mg and K may also be a more effective deterrent to water foragers than a single salt. However, bees may continue to collect less-favored water if it is the only source available (Fig. 4).

### Individual salt preferences

The relationship of bee PER responses to different salt solutions and actual foraging preferences should be examined in future studies. Water collection has a genetic basis (Kryger et al., 2000). We hypothesize that, like sucrose response thresholds (Page et al., 1998), salt preferences will also have a genetic basis that could lead to foraging specializations. In our study, bee identity accounted for a significant ( $P < 0.0001$ ) and substantial 32% of model variance. We assayed salt preferences of bees that foraged at a low-salt water source, but some foragers may exhibit specializations for higher salt concentrations. PER assays of salt preferences may therefore be a useful tool for exploring this neglected aspect of honey bee foraging.

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

J.C.N. and P.W.L. conceived of and designed the experiments. P.W.L. performed the experiments. J.C.N. analyzed the data. J.C.N. and P.W.L. jointly wrote the paper.

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